different pattern sizes and variations in the microstructure, respectively. Here we report on the advantages of PDMS-alternatives for an improved and fast micro-contact printing process. Using those materials we could print proteins in various structures (including pillars, lines and grids) and sizes down to 100 nm. For characterization of pattern quality we used TIRF and super-resolution microscopy and focused on the validation of well-known protein-protein interactions including the one of EGF-, Insulin/IGF1- or beta-adrenergic receptors with intracellular binding partners. As TIRF microscopy requires homogenously adhered cells on the micro-patterned surface, we especially focused on the verification of cell adhesion efficiency when using different pattern shapes.
An important issue which we are currently addressing is the production of a micro-structured and functionalized multiwell plate (96 and 384 well design). This development step will set a milestone concerning the throughput rates of the micro-patterning assay and increase the number of potential users interested in this methodology.

2425-Pos Board B562
Millisecond Time Resolved Electrochemical Detection of Non-Electroactive Neurotransmitter Release
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Acetylcholine and glutamate are highly important non-electroactive neurotransmitter in the mammalian central nervous system. A fast, sensitive method to detect the release of acetylcholine and glutamate at the surface of a single cell is needed to gather data about the kinetics of exocytosis events in pathways involving these signaling molecules.
To this end, carbon fiber electrodes have been modified with electrodeposited gold nanoparticles to increase the effective electrode surface area and provide a high curvature surface for enzyme attachment. For detection of acetylcholine, acetylcholine esterase and choline oxidase were deposited onto the nanoparticle coated electrode surfaces to catalyze acetylcholine to hydrogen peroxide for electrochemical detection. The functionalized electrodes have been characterized to determine the KM and Vmax of the enzymes as well as the total enzyme coverage and gold nanoparticle surface area. This information was further used to evaluate the conditions for optimal retained enzyme activity of the sensor surface. Similarly, glutamate oxidize was placed onto the surface of electrodes plated with nanoparticles. The sensors were tested for acetylcholine and glutamate release from a synthetic cell model for exocytosis, and providing time resolved detection of single vesicle release events on the order of millisecond time scale.

2426-Pos Board B563
Solid-State Nanopore Detection of Epigenetic DNA Modifications
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We describe protein-facilitated solid-state nanopore detection of dsDNA containing single modified nucleotides. We first use model mono-biotinylated oligonucleotides to determine the detection limits of the assay by systematically studying the effect of DNA length and biotin position to better understand the detection process. We then investigate epigenetic modifications by combining selective biotinylation of target modified bases and a charged, high-affinity protein tag to induce translocation events. We use this approach to resolve two major epigenetic modifications: methylcytosine (5mC) and hydroxymethylcytosine (5hmC).

2427-Pos Board B564
Dynamics and Energy Contributions for Transport of Pertactin through an Aerolysin Nanopore
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Autotransporters are a large family of extracellular monomeric virulence proteins from Gram-negative bacteria. Despite their simplicity, many aspects of the autotransporter secretion mechanism remain unclear. We are using pertactin, an archetypical autotransporter from Bordetella pertussis, as a model for secretion studies. The final step of autotransporter secretion is C-to-N-terminal transport of the central passenger domain through the outer membrane, mediated by the C-terminal translocator domain. Passenger folding occurs only after this final secretion step, which requires neither ATP nor a proton gradient. Passenger folding may therefore serve as a driving force for pertactin secretion. For this reason, it is interesting to consider how autotransporters are secreted through their own translocator domain to the cell surface.
As a first step, we are mimicking this transport using a simpler model consisting of a well-known nanopore. Transport of the pertactin passenger is detected at the single molecule level using electrophysiological techniques. We show that unfolded pertactin dynamics through a single aerolysin pore can be described using a model developed for an unrelated protein. A Van't Hoff-Arrhenius law describes the frequency of blockades as a function of the applied voltage. The unfolded chains are dominated by an activation energy that has both an entropic component and an enthalpic origin. We compare our experimental results to theory and show that proteins cross the membrane by passing through the aerolysin nanopore. We have used these results to develop a general description of the comportment of an unfolded protein during its transport through a protein nanopore.

2428-Pos Board B565
A New Environmental Biosensor for Cell Free Synthetic Biological Systems
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Biosensors utilize fundamental properties of biophysics to enable detection of environmental analytes. While these systems are frequently based on living cells, they have potential applications in multiple fields. Cell-free synthetic biological systems, such as artificial cells (i.e., liposomal encapsulations of functional biological parts), are an example of systems that could be enhanced by new biosensors. Here, we created a biosensor based on the biophysical interactions of biotin and streptavidin. Biotin plays an essential role in cell growth and the typical amount of biotin required by cells is low (e.g., 1 ng/ml in *E. coli*). Biotin is also widely used in molecular assembly because of its strong conjugation to streptavidin, with a K_d around 10^{-15} M). We leveraged this strong attraction in a competitive binding scheme to create a biotin sensor that is both specific and sensitive in comparison to common biotin assay methods that are based on radioactive labeling, microbiological, or physicochemical principles. This new biosensor has a detection limit in pg range, and significantly discriminates between biotin and its metabolic precursor in *E. coli*, dethiobiotin. This engineered biosensor can be used as a biotin detector for biotin synthesis by engineered cells. Additionally, it can be deployed in cell-free synthetic systems. Ultimately, our engineered biosensor can be coupled to cell-free systems to act as an environmental reporter. Alternatively, it can trigger cell-free gene expression in artificial cells. We anticipate this new technology will impact work in fields ranging from synthetic biology to the biophysics of biomaterial assembly.
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2429-Pos Board B566
Nanopore Sequencing of "Alien" DNA Bases
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Recently, it was shown for the first time that Escherichia coli can efficiently propagate a genetic alphabet expanded from the four canonical DNA nucleotides to include the synthetic ''alien'' bases d5SICS and dNaM. Traditional sequencing platforms cannot detect these alien bases. We tested whether nanopore sequencing with the protein pore Mycobacterium smegmatis porin A (MspA) is sensitive to d5SICS and dNaM. In nanopore sequencing, a nanometer scale pore provides the only electrical connection between two electrolyte solutions. An applied voltage causes a current to flow through the pore. Negatively-charged single stranded DNA is drawn through the pore, causing a nucleotide specific reduction in the measured current. Thus-far, MspA is the only nanopore shown to demonstrate single-nucleotide sensitivity to standard nucleotides as well as methyl cytosine and hydroxymethyl cytosine. We use the φ29 DNA polymerase to regulate DNA motion to single-nucleotide steps. Here, we demonstrate the direct detection d5SICS and dNaM with MspA-enabled nanopore sequencing.

2430-Pos Board B567
Robust Membrane-Embedded phi29 Motor Channel for Sensing of Single Molecule and High-Throughput Fingerprinting of DNA
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The ingenious design of the bacteriophage phi29 DNA packaging motor with an elegant channel has inspired its applications in nanotechnology. The hub of the motor is a truncated cone shaped connector consisting of twelve protein subunits that form a ring with a central 3.6-nm channel that acts as a path for

dsDNA to enter during packaging and to exit during infection. The connector inserted in lipid bilayer exhibits robust properties and generates extremely sensitive conductance signatures. DNA and RNA can be electrophoretically driven through the channel in a concentration and voltage dependent manner. Information about the structure, length and conformational dynamics can then be deduced by their characteristic dwell times during translocation and by their relative percentage in current blockades. This motor channel further exercises a one-way traffic property for dsDNA translocation from N- to C-terminal with a natural valve mechanism in DNA-packaging. We also demonstrated its utility as a highly sensitive device for capture and fingerprinting of chemicals and biopolymers in real time at extremely low concentrations and in the presence of many contaminants. The phi29 motor channel has potential applications in high-throughput single-pore DNA sequencing, environmental surveillance, athlete drug monitoring, toxin/drug screening, and earlier disease diagnosis.
Supporting publications from Guo lab: (1) *Nature Nanotechnology*. 2009. 4: 765; (2) *Nano Letters.* 2010, 10: 3620; (3) *Molecular Biosystems*. 2010. 6:1844 (4) *Biomaterials.* 2011. 32:8234; (5) *Biophysical Journal.* 2012. 102:127; (6) *ACS Nano.* 2012. 6:3251; (7) *Nature Protocols.* 2013. 8:373; (8) *Nano Today.* 2013. 8:56; (9) *ACS Nano*. 2013. 7:9814.

2431-Pos Board B568
Single Molecule Nucleic Acid Sensing in an Optical Nanopore Array
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By optically encoding the Ca^{2+} flux we are able to parallelize the detection of nucleic-acid binding events in nanopores. We report parallel recordings at a density of ~ 10k measurements per square milimeter in a single droplet hydrogel bilayer (DHB). Both static DNA blockage and kinetic miRNA unzipping events can be monitored optically for single molecule nucleic acid identifications. Sub-pA equivalent amplitude resolution and 3 ms temporal resolution is demonstrated, which enables discrimination between nucleic acids with 1-4 bases difference. To further expand this platform, hydrogel hydrogel bilayer array (HHBa) is formed with micro-patterned hydrogel chip, which is also compatible with a spotting robot for biological screening applications. Based on the enzymatic ratchet speed (~35 Hz), this optical recording platform should produce sequencing signal with a rate of 1 million nucleotides per square milimeter per second, which paves the way to 15 minutes human genome sequencing and other general applications of single molecule sensing with nanopores.

2432-Pos Board B569
An ATR-FTIR based Immuno-Biosensor for the Detection and Analysis of Disease Related Biomarkers from Liquid Samples
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The research and detection of biomarker candidates and their structural information analysis have become clinically important during the last decades. Especially neurodegenerative disorders like Alzheimer's or Parkinson disease are characterized by misfolding of body-own proteins into oligomeric and fibrillar ß-sheet enriched higher-level clusters. According to the detection of specific biomarkers in complex media, enzyme-linked immunosorbent assays (ELISA) demonstrate a high specificity and are sensitive to minute peptide amounts. On the other hand, FTIR spectroscopy has been proved to be quite useful for the detection and analysis of protein secondary structures and conformational changes during disease progression. Particular the attenuated total reflection (ATR) technique has been provided, due to its possibility for surface modification, the selective detection of soluble membrane anchored disease related proteins. Thus, secondary structure analysis of various disease related proteins like Prion Protein (PrP) or Amyloid-beta- (Aß) is achieved.
Here, we demonstrate an ATR-FTIR based biosensor that combines the advantages of both ELISA and FTIR. Thus, we achieve the maximum specificity with simultaneous structure sensitivity in one sensor. Thereby disease related proteins like Amyloid-beta or alpha-synuclein were detected in complex solutions like *cerebrospinal fluid* or blood plasma. The determined secondary structure gave information concerning disease state or progression. However, the immuno-biosensor demonstrates the potential of the FTIR-spectroscopy in the biomedical sector.

2433-Pos Board B570
Slowing Down DNA Translocation and Neutral Single Molecules Detection through Solid-State Nanopores by Pressure
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Charged single molecules of DNA can be detected and characterized with a voltage-biased solid-state nanopore immersed in an electrolyte solution. This has stimulated intense research towards understanding and utilizing this nanosensor device for the analysis of a wide variety of charged polymer molecules, and for the ultimate goal: DNA sequencing. As one of its fundamental challenges, DNA translocation speed through solid-state nanopores (~30 base/us) is too fast for instruments to "read" each base signal compared to their protein counterparts. By taking advantage of the ability of solid-state membranes to sustain large pressure drops without breaking, we show here that a pressure-induced fluid flow, in and near the nanopore, provides an additional force to control the motion of the molecule through the pore. This pressure-derived force, combined with the voltage bias, enables solid-state nanopores to detect and characterize very short molecules, and near-neutral molecules. For uniformly charged polymers like DNA, the pressure-derived force can be countered by the voltage-derived force to slow the molecule motion without reducing the ionic current signal. Modest pressures applied to a voltage-biased nanopore greatly extend their utility as single molecule detectors by enabling neutral molecule capture and detection, as well as control of molecule translocation speeds through the pore. We demonstrate nearly an order-of-magnitude improvement in length discrimination. This broader range of detectable molecule sizes, charge states, and spatial conformations considerably expands the applicability of nanopore detection technologies.

2434-Pos Board B571
A Next Generation Label-Free PoC Sensing Platform
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Current Point-of-Care Testing (PoCTs) or biomarkers are affinity based and require immobilisation on the substrate for sensitive detection. This not only increases the production and overall costs but also introduce measurement error leading to false interpretations. The PoCT devices are well established for protein and cell based assays but challenges remain for label-free, inexpensive and multiplex protein screening tools. Nanopore sensing allows detection of biomolecular interactions and intramolecular structural alterations. It monitors ionic currents changes when a charged molecule translocates through the pore with external electric potential. The ultimate advantage is removing the clouding of ensemble averaging.
There are two types of nanopores, biological and solid-state which both efficiently probe analytes at the single molecule level but they either have limitation on pore diameter or required very expensive equipment to fabricate the pore. Here we employ a conical solid-state nanopore - nanopipettes (sub-nanometer size) to screen through different targets. They are quicker and cheaper to fabricate and can select the optimum pore diameter. The material of nanopipette (quartz) have low electrical noise which would be ideal to differentiate the binding signal with the complex. Traditional assays use antibodies because of high specificity and selectivity however they are difficult to implement onto the nanopipette due to relatively large size and hydrodynamic complexity in the nanochannels. Distinguishing between binding and transient blockade remains unresolved. Aptamers are single-stranded oligonucleotides which bind to relevant target molecules with high affinities similar to antibodies but are more robust, smaller in size and cost effective.
Due to the specificity of the aptamers, integrating with nanopipette will allow single protein molecules to be detected to in a low cost, label-free manner and able to screen targets in a high-throughput format leading to next generation PoC electrochemical sensing platforms for screening proteins.

2435-Pos Board B572
Real-Time Detection of Lipid Bilayer Assembly and Detergent-Initiated Solubilization
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The cellular membrane governs numerous fundamental biological processes, and therefore, developing a comprehensive understanding of its structure and function is critical. However, because of its inherent complexity, this challenge is as yet unsolved. In an attempt to develop a model, two different experimental approaches are being pursued in parallel: performing single cell experiments (top down) and using biomimetic structures (bottom up), such as lipid bilayers. One challenge in many of these experiments is the reliance on fluorescent probes for detection. In the present work, we have used a label-free detection method based on an evanescent optical sensor known as an optical resonant cavity. In this approach, we are able to detect the self-assembly and solubilization of lipid bilayers in real-time. Specifically, using these silica devices, there are two independent detection mechanisms which are able to confirm the formation and detergent assisted solubilization of the lipid bilayers: 1) a refractive index change and 2) a material loss change. Both mechanisms can be monitored in parallel, on the same device, thus allowing for cross-confirmation of the results. To verify the proposed method, we have detected the formation of self-assembled phosphatidylcholine lipid bilayers from SUVs on the device surface